REMARKS

Claims 14 and 23 have been presently amended. Claims 24-26 are new. Amendments to claim 14 were effected primarily to improve clarity and to correct antecedent basis; additional support can be found at, e.g. page 5, lines 17-20. Support for the amendments to claim 23 can be found on page 11, lines 29-30; page 14, lines 31-32; TABLE 1 and FIG. 2A in the application as filed. Support for new claim 24 can be found on page 13, lines 12-13 and FIG. 2; support for new claim 25 can be found on page 13, lines 37-38 and FIG. 1; support for new claim 26 can be found in TABLE 1. No new matter has been introduced by these amendments. Accordingly, entry of these amendments is respectfully requested.

I. Election/Restrictions

Since the Applicant has perfected their foreign priority claim to July 3, 2003, thereby removing the previously cited Schuh *et al.* disclosure from the prior art, the Examiner has presently withdrawn the earlier restriction requirement, acknowledging the unity of invention in the present application. Applicant thanks the Examiner for the kind attention given to this point.

II. Rejection of Claim 23 under 35 U.S.C. §112, ¶2

Claim 23 has been rejected for reciting the limitation "identifying a mutation or a post-translational modification of a gene encoding the PMCA4 isoform according to claim 14", wherein this limitation appeared to lack sufficient antecedent basis from claim 14 (Office Action, page 2).

Applicant has presently effected amendments to claim 23, which now points out and distinctly refers to performing a diagnostic method that includes a step of analyzing a biological sample for aberrations pertaining to the PMCA4 isoform recited by claim 14, wherein such aberrations are diagnostic of infertility in a human male. In particular, the claim recites detecting a mutation or polymorphism in a PMCA4 gene encoding the PMCA4 isoform, or detecting a decrease in the expression of the PMCA4 isoform in the sperm cells relative to a control sample. The technical implementation of both diagnostic steps is clearly explained in

the specification (e.g. as stated above, on page 11, lines 29-30; page 14, lines 31-32; the EXAMPLES, TABLE 1 and FIG. 2A) such that the skilled person receives sufficient guidance to readily practice the subject-matter of the invention.

Accordingly, Applicant respectfully requests that the present rejection under 35 U.S.C. §112, ¶2 be reconsidered and withdrawn.

III. Rejection of Claim 23 under 35 U.S.C. §112, ¶1

Claim 23 has been rejected as not being enabled for a diagnostic method for determining infertility in a human male "comprising identifying a mutation or a post-translational modification of a gene encoding the PMCA4 isoform referred to by claim 14" (Office Action, pages 3-7).

As explained in the previous section, Applicant has amended claim 23 (and submitted new associated-dependent claims 24-26) to recite diagnostic methods which are clearly enabled by sufficient guidance in the application as filed, such that the skilled person can readily practice the inventive method over the entire scope claimed, without the need for any undue experimentation. For example, claim 23 is presently directed to analyzing the PMCA4 isoform in a biological sample containing one or more sperm cells using exemplary methodology as described in the specification, for example: (1) a scheme for creating and analyzing PMCA4-deficient knockout mice is provided in FIG. 2, wherein sperm cells derived from these mice were demonstrated to be infertile (FIG. 2C) and have a high percentage of non-motile sperm compared to normal controls (86% v. 27%, respectively, page 15, lines 30-34); and (2) a decrease in the expression of the PMCA4 isoform in the sperm cells can be readily detected using an expression analysis, as described on page 13, line 25 to page 14, line 6, with results being depicted in FIG. 1.

At least for these reasons, Applicant respectfully submits that one skilled in the art, in view of the provided technical disclosure of the instant invention, with the supporting working Examples, clearly associates a compromised PMCA4 activity and/or expression in sperm cells with a *commensurate decrease* in sperm motility that leads, e.g. to infertility in a male. Hence,

because the claimed embodiment is clearly described, and operable, Applicant respectfully requests that the Examiner reconsider and withdraw this rejection under 35 U.S.C. §112, ¶1.

IV. Rejection of Claims 14-15 and 17-20 under 35 U.S.C. §103: Chaudhary + Wennemuth

Claims 14-15 and 17-20 stand rejected as being unpatentable over Chaudhary *et al.* ("Chaudhary") in view of Wennemuth *et al.* ("Wennemuth"). Applicants respectfully traverse this rejection (Office Action, pages 7-10).

Brief Overview of the Claimed Invention:

In the instant invention, Applicant has identified the numerous disadvantages associated with conventional contraception methods, including known side-effects associated with hormonal contraception (page 1, line 30 to page 2, line 12), the unreliability of "natural contraception" methods (page 2, lines 14-17) and complications commonly occurring with mechanical methods such as the IUD device. Accordingly, the invention provides, for the first time, technical information that clearly demonstrates: (1) the expression of plasma membrane calcium ATPase 4 (PMCA4) in sperm (in *both* the tail/flagella *and* the *acrosomal* portion) (see FIG. 1 and page 3, lines 7-16), (2) infertility in PMCA4-knockout mice (FIG. 2 and page 3, lines 18-29), (3) direct suppression of sperm mobility by PMCA4 inhibitors in sperm (see page 14, from line 8 and TABLE 1) and significantly, (4) comparative data showing a pronounced decrease in sperm mobility for *both PMCA4-deficient sperm* (in the knockout mice) and *in sperm cells subject to a treatment with PMCA4 inhibitors* (page 15, from line 15) when compared to control samples. Collectively, the invention provides new information regarding the physiological localization and functionality of the PMCA4 isoform in mammalian sperm cells, with compelling empirical data that supports a new contraceptive option (and infertility testing parameter) to thus overcome many of the known disadvantages in this technical field.

Chaudhary:

The Chaudhary disclosure relates to the characterization of Caloxin as an extracellular plasma membrane Ca2+-ATPase (PMCA) pump inhibitor, specifically on the PMCA1b isoform, in the sarcoplasmic reticulum localized in skeletal muscle (Abstract). The Examiner notes that

PMCA sequences are "conserved in the various isoforms", Chaudhary explains that this might be true "[e]xcept for the first putative domains" (p. C1027, right-col.). Furthermore, **Chaudhary states that PMCA isoform 1b is distinct from PMCA4** in that the "residue A is replaced by P" in one of the extracellular domains (p. C1029, left-col.; emphasis added), which could profoundly influence the binding/affinity of PMCA4 for particular ligands, when compared to PMCA1b—*especially* since residue **A** (alanine) is characterized by a simple aliphatic chain while residue **P** (proline) has a distinctive cyclic structure in a side chain that locks its ϕ backbone at a dihedral angle at approximately -75°, giving proline an ***exceptional conformational rigidity*** compared to other amino acids. This could certainly impact how PMCA4 interacts with, e.g. an inhibitor for PMCA1b or with any other PMCA isoform—however, we are not given data on this topic.

Therefore, it is not clear that "the PMCA inhibitor of Chaudhary would necessarily inhibit PMCA4", as the Examiner states (Office Action, page 8; emphasis added), since Chaudhary admits that "**one cannot rule out different affinities of Caloxin 2A1 for individual [PMCA] isoforms**" (p. C1029, left-col.; emphasis added). Moreover, Chaudhary observes that Caloxin 2A1 inhibits PMCA4 in "erythrocyte ghosts" "expressing *mainly* [but not exclusively] PMCA4", but, in view of the above admission, it is certainly possible that Caloxin is binding to another PMCA isoform in the ghosts with higher affinity / specificity—we can't be sure without additional experimentation. This latter observation is important, in view of the admitted differences between the PMCA1b and the PMCA4 extracellular domain sequences. In addition, the skilled person, without further information, would not be able to safely conclude that Caloxin would behave in the same way in a sperm cell, a cell type that was not considered by Chaudhary (more on this point below).

Moreover, Chaudhary restricts his experimental methodology to the use of Caloxin against the "second putative extracellular domain of PMCA1b in rabbit" having the specified residues (p. C1028, "*Methods*", left-col.). Given the admitted unknowns that exist between the PMCA isoforms and their ability to bind Caloxin, Applicants submit that no general conclusion regarding Caloxin-PMCA behavior can be drawn without undertaking experiments to confirm this hypothesis, even if certain of the binding domains in these isoforms *might be* conserved.

Also, Applicant is not clear how inhibition of PMCA1b in the skeletal muscle, or the mixture of PMCAs that exist in the erythrocyte ghosts, can be automatically extended to *every other type of mammalian tissue* considering present inventor's observations confirming that: (i) the "transcription of the PMCA splice variants for these [PMCA] gene products is **tissue-specific**" (Specification, page 4, lines 31-32; emphasis added); and (ii) the different PMCA splice variants "have different affinities for **calcium** and calmodulin" (Specification, page 4, lines 35-37; emphasis added). In fact, the Chaudhary disclosure acknowledges this point, stating that:

> The PM Ca^{2+} pump affects virtually every cell, **although its role in cell function varies**, depending on the expression and level of activity of other transporters. Obviously, this discovery **paves the way to examine the role of PM Ca^{2+}** in tissues with high levels of PMCA expression such as..." (N.B. an extensive list of tissues and cells is mentioned, but NOT sperm cells, suggesting Chaudhary *did not know* about the existence of PMCA in these cells) (p. C1029, right-col.; emphasis added).

Hence, Applicants are unclear precisely how the PMCA inhibitors used in the Chaudhary models would *necessarily inhibit* the PMCA4 isoforms found in the sperm cells tested in the presently claimed invention.

Wennemuth:

The Examiner acknowledges that Chaudhary does not "specifically teach a method achieving a contraceptive effect comprising a PMCA4 isoform inhibitor or a method for diagnosing infertility in a human male" (Office Action, page 8). To satisfy this deficiency, the Examiner refers to Wennemuth, which relates to a comparative study of Ca^{2+} clearance mechanisms in mature sperm by, e.g. changing the ion content of the medium using depolarization events to examine calcium recovery (p. 116, left-col.). In particular, two distinct types of Ca^{2+} channels, **the PMCA and NCX channels** were evaluated and, as explicitly noted by Wennemuth, *both* found to "dominate rapid Ca^{2+} clearance" (p. 118, left col.; FIG 2/PMCA & FIG. 4/NCX), wherein *both* Ca^{2+} channels were observed to act in "**synergy**" during the Ca^{2+} clearance process (p. 120, left-col.) in the mouse sperm. Hence, the skilled person would be unable to attribute Ca^{2+} clearance, rapid or otherwise, exclusively to the PMCA channels, and would not know if inhibition of these channels alone would render sperm cells non-motile.

The Examiner notes that Wennemuth thought the PMCA "performs the major task of Ca^{2+} clearance" (Office Action, page 9). However, as mentioned above, *following* the experiments, Wennemuth concludes that "[e]vidently a **significant NCX is present**" (p. 119, right-col. and FIG. 4), wherein the obtained $[Ca^{2+}]_i$ nM values ***are similar to*** those observed for the **PMCA** (see FIG. 2). Moreover, Wennemuth concludes that **"[t]ogether** the PMCA and the NCX are dominant routes of Ca^{2+} clearance in sperm" and significantly, that "some residual clearance mechanism still operates in Li8.6" during the depolarization event, e.g. in FIG. 6 (paragraph bridging pages 120-121; emphasis added). Considering the above information, the skilled person could not firmly conclude precisely *which of the two* (or perhaps three) Ca^{2+} channels are responsible for the Ca^{2+} clearance, such that inhibition of said channel *would necessarily result in a contraceptive effect* (Office Action, page 10).

The Examiner states that the "inhibition of PMCA would necessarily result in a contraceptive effect as the acrosome reaction (i.e. sperm penetrating the egg or ovum" would necessarily be prevented; Office Action, page 10) in view of this receptor's purported role in Ca^{2+} regulation in sperm cells. However, and to emphasize, Wennemuth discloses that there are four major Ca^{2+} clearance mechanisms in "most animal cells" (PMCA, NCX, SERCA and MCU; page 115, right-col. and in the Office Action, page 9), and as stated above, *at least two* different Ca^{2+} clearance mechanisms acting synergistically in sperm cells (and possibly a third, see FIG. 9A/as re-published). Additionally, the Western blot analysis in FIG 8D, which attempts to provide information regarding PMCA receptor localization, uses 'Antibody 5F10' for PMCA channel detection—an antibody that is non-specific for a particular PMCA isoform (p. 116, right-col, "*Materials*"). Therefore, Wennemuth does not provide the skilled person with sufficient evidence permitting a foregone conclusion that inhibiting PMCA channels alone (and which isoform?) is sufficient to necessarily result in a contraceptive effect.

Moreover, Wennemuth acknowledges that related studies have implicated an additional Ca^{2+} regulatory role for T-type Ca^{2+} channels in "rapid" changes in $[Ca^{2+}]_i$ as detected by techniques offering "higher time resolution"— perhaps due to a "readjustment of local gradients" rather than to the classical clearance of global Ca^{2+} regulated by, e.g. the PMCA and

NCX transporters (p. 125, left-col.). Wennemuth also appreciates that there is still much work to be done to fully understand "the compartmentalization of Ca^{2+} effects in the sperm and how local sites of entry may be couple with local sites of action and clearance" (p. 126, left-col.). Thus, the skilled person would immediately realize that there are many open questions about precisely how any of the above-described Ca^{2+} channels can be useful as a clinical target of any kind in the sperm cell.

The combination of Chaudhary and Wennemuth does not render the present invention as obvious to the skilled person:

Applicants consider that in view of these cited disclosures it is not, in fact, obvious to the skilled person to apply information from a study describing the inhibition of the PMCA1b isoform via Caloxin to the admittedly distinct PMCA4 isoform in a completely different cellular environment, due to the *known differences* in the tissue-specific expression, reactivity, structure and ligand affinity of the various PMCA isoforms.

Nowhere does Chaudhary refer to sperm cells, or perform experiments specifically examining the efficacy of Caloxin in PMCA4 sperm cell motility inhibition. And, Wennemuth only considers the PMCA family as a whole in the described depolarization studies: no individual PMCA isoform is accounted for. Wennemuth also describes the synergy between PMCA and NCX channels in the Ca^{2+} clearance process (with *possible participation* from an unknown channel and perhaps the T-channel). Thus, at the Wennemuth publication date, a skilled person could not conclude that exclusively blocking the "PMCA" channels alone (whichever isoform that might be) would be sufficient for / necessarily result in a contraceptive effect or infertility—these endpoints were never investigated by either Wennemuth or Chaudhary.

Consequently, independent claims 14 (and associated-dependent claims 15 and 17-19, which incorporate the features thereof) and 20 are not *prima facie* obvious over Chaudhary in view of Wennemuth, at least because: the admitted uncertainty in the art regarding the tissue-specific expression and ligand binding behavior of the PMCA family; the failure of either disclosure to describe PMCA4 as a lead target molecule in sperm for preventing conception or

inhibiting fertility; no specific motivation to combine these disclosures as the combination of features cannot be done with a reasonable expectation of success (since the skilled person could, at best, only *presume* that a simple substitution of PMCA4 for PMCA1b and/or all PMCAs isoforms would predictably work in the instant invention as claimed, despite indications that a blanket interchangeability between the two isoforms is not appropriate).

Accordingly, since *prima facie* obviousness has not been established, Applicant respectfully requests that the present rejection under §103(a) be reconsidered and withdrawn. Additionally, Applicant submits that claims 24-26 which inherit the limitations of claims 23 and 14 are not obvious in light of the cited art.

V. Rejection of Claim 16 under 35 U.S.C. §103: Chaudhary + Wennemuth + Zimmermann

Claim 16 is rejected as being unpatentable over Chaudhary, Wennemuth and Zimmerman *et al.* ("Zimmermann"). Applicants respectfully traverse this rejection (Office Action, pages 10-11).

The teachings of Chaudhary and Wennemuth are set forth above. As discussed, both of these publications fail to guide the skilled person to the teachings of the present invention; before the invention's priority date, the specific physiological significance of PMCA, in particular the PMCA4 isoform, on the fertility of sperm was not known. The use of PMCA4 inhibitors to specifically target and suppress sperm mobility, in combination with observations that the knockout of the PMCA4 gene rendered complete infertility—were nowhere disclosed or suggested by the cited references. Neither Chaudhary nor Wennemuth actually performed these experiments, thus no firm conclusions of how the Chaudhary + Wennemuth composition would function *in vivo* can be drawn.

The Examiner observes that Zimmermann teaches "male contraceptive composition[s]" that can be administered orally, parenterally, or topically, and that it would have been obvious to the skilled person to apply such modes of administration to the "PMCA4 inhibitor composition of Chaudhary" to thus render the claimed invention as obvious to the skilled person (Office Action, page 11).

Again, Applicants note that Chaudhary and Wennemuth, alone or in combination with publicly available knowledge at the documented invention date, fail to yield a set of elements that *predictably yield* the claimed invention, with a reasonable expectation of success. Because, as discussed above, Chaudhary does not, in fact, conclude that PMCA1b can be readily exchanged with PMCA4 in a predictable fashion, and is silent on whether PMCA4 is localized in sperm cells, the skilled person can't be certain that Chaudhary provides a "PMCA4 inhibitor composition" without further investigation. Since Wennemuth suggests multiple types of Ca^{2+} channels play a role in sperm Ca^{2+} regulation, the skilled person couldn't predict that PMCA (specifically, PMCA4) was solely responsible for the observed effects, wherein its inhibition would automatically result in sperm non-motility. Zimmermann fails to provide any teaching to remedy these deficiencies.

Since *prima facie* obviousness has not been established, Applicant respectfully requests that the present rejection under §103(a) be reconsidered and withdrawn.

VI. Rejection of Claims 21-22 under 35 U.S.C. §103: Chaudhary + Wennemuth + Papurt

Claims 21-22 are rejected as being unpatentable over Chaudhary, Wennemuth and Papurt *et al.* ("Papurt"). Applicants respectfully traverse this rejection (Office Action, pages 12-13).

The teachings of Chaudhary and Wennemuth are set forth in detail above. As stated, neither of these publications provides the skilled person with a set of features, from a finite set of solutions, that offer both predictability and a reasonable expectation of achieving the claimed invention without undertaking a full research program, i.e. to consider the diversity of available PMCA isoforms, their localization, and their precise role in sperm motility that could be useful for the development of a contraceptive solution.

The Examiner notes that Papurt is directed to "the use of condoms and contraceptive devices as mechanical barriers" "to prevent contraception", including condoms (Office Action, page 12) and further that it would have been obvious for the skilled person to "add the use of

condom to the modified contraceptive method of Chaudhary", and would have been motivated to do so in order to provide an "enhanced composition method" (Office Action, page 13).

Applicants emphasize that since Chaudhary and Wennemuth, alone or in combination with publicly available knowledge at the documented invention date, fail to yield a set of features that *yield* the contraceptive methods and compositions described and claimed in the present invention; the Papurt approach can not be reasonably found to motivate the skilled person to implement a variation, i.e. adding a conventional contraceptive and/or condom to thus arrive at the same contraceptive compositions as those presently claimed.

Since *prima facie* obviousness has not been established, Applicant respectfully requests that the present rejection under §103(a) be reconsidered and withdrawn.

VII. CONCLUSION

In view of the amendment and arguments set forth above, Applicants consider that the objections and rejections in the Office Action mailed on April 16, 2009 have been overcome. Accordingly, we respectfully request that the Examiner issue a Notification of Allowance.

In the event that the Examiner maintains any of the rejections under 35 USC §103, the Examiner is respectfully requested to restate the rejection, particularly in view of statements made herein seeking clarification and in view of the pending claims as presented and/or amended.

If the Examiner believes that a telephone conference would expedite the allowance of the present case, or has any questions or concerns regarding this Amendment, Applicants would welcome a telephone call to Applicant's undersigned attorney at any of the numbers indicated below.

Respectfully submitted,

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